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REGULAR ARTICLE

# Dynamics of plant metal uptake and metal changes in whole soil and soil particle fractions during repeated phytoextraction

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**Abstract**
*Aims* Phytoextration of metal polluted soils using hyperaccumulators is a promising technology but requires long term successive cropping. This study investigated the dynamics of plant metal uptake and changes in soil metals over a long remediation time.
*Methods* A soil slightly polluted with metals (S1) was mixed with highly polluted soil (S4) to give two intermediate pollution levels (S2, S3). The four resulting soils were repeatedly phyto-extracted using nine successive crops of Cd/Zn-hyperaccumulator *Sedum plumbizincicola* over a period of 4 years.
*Results* Shoot Cd concentration decreased with harvest time in all soils but shoot Zn declined in S1 only. Similar shoot Zn concentrations were found in S2, S3 and S4 although these soils differed markedly in metal availability, and their available metals decreased during phytoextraction. A possible explanation is that plant active acquisition ability served to maintain plant metal uptake. Plant uptake resulted in the largest decrease in the acid-soluble metal fraction followed by reducible metals. Oxidisable and residual fractions were less available to plants. The coarse soil particle fractions made the major contribution to metal decline overall than the fine fractions.
*Conclusion Sedum plumbizincicola* maintained long term metal uptake and the coarse soil particles played the most important role in phytoextraction.

**Keywords** Zinc/Cadmium · Hyperaccumulator · Successive cropping · Particle-size · Metal availability

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## Introduction

Metal pollution of some soils has become a serious environmental concern and a potential threat to human health. The technique of phytoextraction by hyperaccumulators has been shown to be successful in remediating soils polluted with metals (McGrath et al. 2006; Japenga et al. 2007). However, the technique

requires long periods of remediation effort and successive crops are needed to remove adequate amounts of metals from contaminated soils to achieve safe levels (Koopmans et al. 2008). One of the important factors limiting metal phytoextraction is the availability of the metals in the soil because this controls plant metal uptake (Whiting et al. 2001). The availability of metals to plants in soil usually depends on the soil properties (e.g. soil metal concentrations, pH, and the soil components) (Kirkham 2006) and plant activity such as plant metal uptake and the activity of the rhizosphere (Puschenreiter et al. 2005; Alford et al. 2010). The available fraction of soil metals decreases over a prolonged phytoextraction time (Liu et al. 2011) and this may be a major constraint for successful practical phytoextraction of polluted soils. Short-term remediation is not adequate for predicting the dynamics of plant metal uptake and changes in soil metals during the phytoextraction process. It is therefore desirable to study this process over relatively long time periods.

Numerous soil factors can influence metal availability, notably particle size distribution, mineral components and organic matter, pH and redox status, and the metal concentrations present. Furthermore, soil particle size distribution has often been neglected in remediation studies. Soils can be fractionated into a series of particle size ranges with various chemical and physical properties that play different roles in metal bioavailability. Fine particles have high specific surface areas, producing much more net negative charges on the soil surfaces and larger amounts of silicates, organic matter, and hydroxides of Fe/Al/Mn/Si than coarse particles (Liu et al. 2006). These different properties will result in different metal adsorption and immobilization capacities when metals enter the soil (Minkina et al. 2011) and will also confer different desorption abilities (Gray et al. 1999). All these different properties among the various soil particle fractions give them distinct capacities to regulate the fate and bioavailability of trace metals in the soil. Numerous studies have shown that the fine particle fractions have higher metal concentrations than coarse fractions (e.g. Lair et al. 2007; Tang et al. 2009), and metals in the clay fraction of urban soils have higher bioavailability than those in other fractions or whole soil (Madrid et al. 2008), but fine clay-size fractions also make Cd less bioavailable when Cd enters the soil (Onyatta and Huang 2006). Most studies have focused on static metal distribution and have evaluated bioavailability using chemical agents. Little is known about changes induced by plant activities in metal availability in different soil size particles.

Field studies have indicated the high potential of using *Sedum plumbizincicola* (*S. plumbizincicola*) for Cd and Zn phytoextraction (Wu et al. 2006, 2008, 2013). This may be due to its high capacity to accumulate Cd and Zn in the shoots, its relatively large biomass, and the absence of visible symptoms of phytotoxicity in soils that are highly polluted with the metals. In the present study, nine consecutive crops of *S. plumbizincicola* were grown on four metal contaminated soils with different pollution levels over a period of 4 years. The amounts of metals removed by the plants and changes in the metals (total and speciation based on a sequential extraction procedure) in various soil particle fractions with and without phytoextraction were determined.

The objectives were to investigate the dynamics of plant Cd and Zn uptake during repeated phytoextraction and to examine the changes in Cd and Zn in whole soil and soil particle size fractions after repeated phytoextraction and to elucidate the role of different size soil particles in phytoextraction. This information will help us to understand the interactions between hyperaccumulators and contaminated soils during phytoextraction and to optimize the efficiency of the phytoremediation process.

## Materials and methods

### Soil properties and repeated phytoextraction experiment

The contaminated soil used in the experiment was a Typic Agri-Udic Ferrosol (Gong et al. 1999). It was collected from the top 15 cm of the soil profile of an agricultural field near a copper smelter in the suburbs of Hangzhou city, Zhejiang province, east China. Two levels of Zn- and Cd-polluted soil were collected, namely lightly polluted soil (S1, with total concentrations of Cd, Zn, Cu and Pb of 1.01, 310, 65.4 and 87 mg $kg^{-1}$, respectively) collected about 150 m from the pollution source and highly polluted soil (S4, with total Cd, Zn, Cu and Pb of 14.7, 6191, 984 and 975 mg $kg^{-1}$, respectively) collected only 30 m from the smelter. The soil pH ($H_2O$) values were 6.47 (S1) and 7.24 (S4). The two soils were air-dried at room temperature, passed through a 2-mm nylon sieve, and mixed thoroughly. The two contaminated soils were then mixed together in proportions

of 2:1 and 1:2 by weight to obtain two intermediate pollution levels (S2 and S3). The resulting four polluted soils were used in the repeated phytoextraction experiment after S2 and S3 were equilibrated for 1 month maintaining water content at 70 % field capacity and then air dried. The main properties of S1, S2, S3, and S4 are listed in Table 1.

The experiment was conducted between 2006 and 2010 in two successive parts. The first part was conducted from 2006 to 2008. Air-dried soil equivalent to 1.5 kg (oven dry basis) was placed in each plastic pot (16 cm upper diameter, 11 cm basal diameter, and 14 cm high), and a plastic tray under each pot was used to water the soil. Two planting treatments (i.e. unplanted control and planted with *S. plumbizincicola*) of each contaminated soil were set up with five replicate pots per treatment. Five seedlings (from seed) were transplanted in each of the planted pots. The first transplant was conducted in April 2006. The plant shoots were harvested (the roots remaining in the soil) when the shoot biomass was adequate and the environmental conditions were suitable for transplanting of the next crop. After the harvest the next crop remediation started. Six successive plant shoot harvests were made in total, and the harvest times were November 2006, April 2007, July 2007, November 2007, and April 2008. The second part was conducted from 2009 to 2010 because some soil in each pot was used for another experiment and the remaining soil from each pot (soils S1, S2, S3, and S4) was used again to continue phytoextraction. In this part of the experiment 0.6 kg (oven dry basis) soil was placed in each pot (10 cm diameter, 13 cm high). The unplanted and planted treatments were continued for each contaminated soil with three replicates and two plants per pot in the planted treatment. The seedlings of the seventh crop were transplanted in August 2009, and three shoot harvests were collected in November 2009, April 2010, and July 2010. In the first crop 100 mg $kg^{-1}$ of N (as $NH_4NO_3$), 80 mg $kg^{-1}$ P and 100 mg $kg^{-1}$ K (as $KH_2PO_4$) were added as basal fertilizer to each pot of the planted treatments and in the remaining crops 100 mg $kg^{-1}$ of N (as $NH_4NO_3$), 50 mg $kg^{-1}$ P and 50 mg $kg^{-1}$ K (as $KH_2PO_4$) were added with 20 ml deionized water to each pot of the planted treatments. During plant growth deionized water was applied daily to the pots to maintain the soil water content at about 70 % field capacity and to the unplanted treatments only deionized water (without fertilizer) was added to maintain the soil moisture as in the planted pots. After the shoots were harvested in each crop the plant roots remained in the soil because these very fine roots could not be separated from the soil. This experiment was conducted in a glasshouse located in Nanjing, east China. The maximum, minimum and average temperatures were 35, 15 and 19–31 °C, respectively. At the end of the experiment the soil from each pot was air dried, mixed, and stored for subsequent analysis. The plants harvested

**Table 1** Selected properties of the soils

| Soil sample | | S1 | S2 | S3 | S4 |
|---|---|---|---|---|---|
| pH | Unplanted | 6.47 | 6.95 | 7.13 | 7.24 |
| | Planted | 4.26 | 4.61 | 4.67 | 6.35 |
| Sand (%) | | 35.1 | 36.6 | 33.4 | 32.0 |
| Silt (%) | | 50.8 | 52.8 | 54.0 | 57.8 |
| Clay (%) | | 14.0 | 6.0 | 12.6 | 10.2 |
| CEC (cmol $kg^{-1}$) | | 12.4 | 12.8 | 11.1 | 10.7 |
| OC (g $kg^{-1}$) | | 42.2 | 37.6 | 33.7 | 29.1 |
| Available-P (mg $kg^{-1}$) | | 88.6 | 90.4 | 137 | 111 |
| Particle mass Unplanted/Planted (g) | 50–250 μm | 42.7(0.4)/42.6(0.2) | – | – | 42.6(0.4)/48.5(0.5) |
| | 5–50 μm | 34.4(0.4)/33.0(0.1) | – | – | 35.2(0.3)/30.3(0.2) |
| | 1–5 μm | 16.0(0.3)/15.4(0.3) | – | – | 15.2(0.7)/14.0(0.1) |
| | 0.1–1 μm | 3.20(0.05)/3.10(0.02) | – | – | 2.94(0.06)/3.95(0.07) |
| | <0.1 μm | 0.58(0.01)/0.89(0.04) | – | – | 0.68(0.22)/0.96(0.09) |

S1, lightly polluted soil; S2 and S3, intermediate polluted soils; S4, highly polluted soil; OC, Soil organic carbon; "–", non-analysis; data shown as mean or mean(SD)

from each crop were washed with tap water and then with distilled water, oven dried to constant weight at 80 °C, and stored prior to metal determination.

### Soil particle size fractionation

The original contaminated soils (S1 and S4) without and with phytoextraction were separated into five different size particle fractions at the end of phyto extraction: fine sand (50–250 μm), silt (5–50 μm), fine silt (1–5 μm), colloidal (0.1–1 μm), and nanocolloidal (<0.1 μm) fractions. The procedures were briefly as follows: (1) air-dried soil was ground and passed through a 250 μm nylon sieve, then 50 g of the sieved soil was mixed with 1 L deionized water and left overnight before ultrasonic dispersion for 10 min at 25 °C. The suspension was slowly passed through a 50-μm nylon sieve and then the fraction retained by the sieve was carefully washed with deionized water until clear. Particles remaining on the sieve were the fine sand fraction. (2) The suspension that passed through a 250 μm nylon sieve was transferred to a 5-L glass beaker. Deionized water was added until the volume of the suspension was 3 L, mixed thoroughly and left to sediment for 74 min at 20 °C and then the upper layer of the suspension (0–10 cm) was decanted off. The sedimentation time was calculated according to Stokes' law (particles >5 μm). The above procedure was repeated until the upper layer liquid was clear. The sediment was regarded as silt. (3) The 1–5 μm fine silt and 0.1–1 μm colloidal particles were fractionated by the centrifuge method to save time and the formula used to calculate the centrifuge time and other details were presented previously by Tang et al. (2009). The decanted liquid from Step (2) was centrifuged, obtaining the pellet (1–5 μm, fine silt) at 1,500 rpm for 5 min, and then the supernatant was centrifuged at 4,500 rpm for 54 min and the pellet was regarded as colloidal particles (0.1–1 μm). (4) <0.1 μm nano colloidal fractions were present in the supernatant from the colloidal fraction at step (3). A cross-flow filtration device (Model A, Tianjin Polytechnic University Motian Group, Tianjin, China) was used to condense the supernatant. The different size fractions obtained at the different steps were evaporated to dryness in an oven at 60 °C. More detailed information about the soil size fractions can be found in the article by Tang et al. (2009). The weights of fine sand, silt, fine silt, colloids, and nanocolloids in 100 g bulk soil are listed in Table 1. The particle recoveries of the test soil were 96.4–97.8 % and the particle metal recoveries relative to whole soil were from 85.9 to 100 % with the exception of high Cd (170 %) and Zn (120 %) recovery in S1 of the planted treatments. The high metal recovery in planted treatment S1 may have resulted from the low metal concentrations in this soil which may have resulted in relatively high analytical error.

### Chemical analysis

### Soil physico-chemical properties

Soil pH was measured with a glass electrode at a soil: water ratio of 1: 2.5. Soil organic carbon (OC) was determined by the Walkley-Black method and total nitrogen by Kjeldahl digestion and distillation. Available phosphorus (P) was extracted with 0.5 M $NaHCO_3$ by the Olsen methods. Soil cation exchange capacity (CEC) was determined by exchange with ammonium acetate (1.0 mol $L^{-1}$, pH 7.0) and titration with HCl. Free iron oxides ($Fe_d$) were extracted with $Na_2S_2O_4$-$Na_3C_6H_5O_7$-$NaHCO_3$ and determined by atomic absorption spectrophotometry (Varian Spect rAA 220FS, Varian, Palo Alto, CA). The mineral composition of the soils and particle fractions was determined semi-qualitatively using an X'pert Pro diffractometer (Philips, Amsterdam, the Netherlands) after the soil samples were ground and passed through a 0.074 mm nylon sieve. The particle size distribution was obtained with a Beckman LS230 laser diffraction apparatus. The chemical properties of soil size fractions are shown in Tables 1 and 2.

### Sequential extraction of metals

Air-dried whole soil and soil particles of S1 and S4 (2.00 g) were used for extraction using the modified BCR (Community Bureau of Reference) sequential extraction procedure following Luo and Christie (1998). In brief: step 1 (Acid-extractable), 20 ml of 0.11 M $CH_3COOH$ shaken for 16 h; step 2 (Reducible): 20 ml of 0.10 M $NH_2OH{\cdot}HCl$ adjusted to pH 2.0 with high purity $HNO_3$ shaken for 16 h; step 3 (Oxidisable), 10 ml of 30 % $H_2O_2$, to avoid losses due to violent reaction, the mixture was allowed to digest in the cold for 1 h with manual shaking and was then taken to dryness in a water bath heated to 85±2 °C. A further 10 ml of $H_2O_2$ were added and taken to dryness in a water bath at 85±2 °C with occasional agitation. After cooling, 25 ml of 1 M

**Table 2** Selected properties of soil particle fractions before phytoextraction

| Soil sample | | CEC (cmol $kg^{-1}$) | $Fe_d$ (%) | OC(g $kg^{-1}$) | Mineral composition (%) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Quartz | Feldspar | Hydromica | Smectite | Attapulgite | Carbonate |
| S1 | Fine sand | 8.12 | 0.76 | 26.7 | 55–60 | 15–25 | 15–25 | – | – | – |
| | Silt | 12.1 | 0.70 | 21.2 | 70–80 | 5–10 | 10–20 | – | – | – |
| | Fine silt | 26.8 | 1.37 | 42.8 | 50–55 | <10 | 10–20 | <10 | <10 | – |
| | Colloids | 36.6 | 1.69 | 57.0 | 15–25 | 15–25 | – | 50-60 | – | – |
| S4 | Fine sand | 6.55 | 0.70 | 26.2 | 65–70 | 10–15 | 15–25 | <10 | – | – |
| | Silt | 5.55 | 0.54 | 14.7 | 65–70 | <10 | 5–10 | – | – | <10 |
| | Fine silt | 21.1 | 0.97 | 36.9 | 45–55 | <10 | 10–20 | <10 | <10 | – |
| | Colloids | 28.1 | 1.35 | 47.4 | 25–30 | 10–20 | 40–45 | – | – | – |

S1, lightly polluted soil; S4, highly polluted soil; $Fe_d$, Free iron oxides; OC, Soil organic carbon

$NH_4OAc$ adjusted to pH 5.0 with $CH_3COOH$ were added to the dry residue and the mixture was extracted by shaking for 16 h; step 4 (Residual): this metal fraction was measured after *aqua regia* extraction. Extractions were carried out on three replicates of the whole soils and soil particles. A certified reference material (BCR701) was included in addition to the soil samples for quality control. The average recoveries of three steps of BCR 701 were 99, 89, and 80 % for Cd, and 90, 93, 90.7 % for Zn respectively and all soil sample recoveries (sum of four fractions/total in soil×100) were between 90 and 120 %.

### Cadmium and zinc determination

Cadmium and Zn in the bulk soil and in the residual fractions from BCR extraction were extracted using *aqua regia*: test portions of 0.25 g were digested with 12 ml of HCl-$HNO_3$ (4:1, v/v). The metals in the extracts were determined by atomic absorption spectrometry (AAS) using a Varian SpectrAA 220FS or 220Z (Varian, Palo Alto, CA). Plant samples (0.25 g) were digested using a mixture of 6 ml $HNO_3$ and 4 ml $HClO_4$ and the metals were also determined by AAS. Replicate samples (parts of the soil particle samples were chosen as replicates for metal analysis because of limited amounts of small size particles), blanks, and a certified reference material (GBW07401, provided by the Institute of Geophysical and Geochemical Exploration, Langfang, Hebei province, China) were included in all analyses for quality control. Cd and Zn concentrations in the BCR sequential extraction were determined by AAS.

### Statistical analysis

Statistical analysis was performed by one-way analysis of variance (ANOVA) with Duncan's multiple range test at the 5 % level to compare the mean values using the SPSS version 16.0 for Windows software package. Data are presented as mean ± standard deviation (SD).

## Results

### Shoot biomass and metal concentrations

During repeated phytoextration the plants did not show any visible symptoms or growth inhibition. Greater shoot biomass was obtained in crops one to six than the last three crops (Table 3). This might be related to the smaller soil volume provided for the last three crops (0.6 kg soil).

Plant Cd and Zn concentrations at each harvest are shown in Fig. 1. Plant Cd increased with increasing soil Cd. During the repeated phytoextraction plant Cd showed a clear decreasing trend but at later stages of phytoextraction plant Cd did not show any decrease, especially in S1 and S4. In the first crop, higher plant Zn was found in S4, but there was no significant difference in plant Zn among S1, S2 and S3. During the repeated phytoextraction plant Zn decreased initially and then remained stable in S1, and increased in the first four crops of S2 and S3, but in S4 similar plant Zn concentrations were found in all crops. Plant Zn in S4 showed no significant difference from that in S2 or S3.

**Table 3** Shoot biomass in each of nine crops during phytoextraction (g dry weight plant$^{-1}$)

| Soils | 1st | 2nd | 3rd | 4th | 5th | 6th | 7th | 8th | 9th |
|---|---|---|---|---|---|---|---|---|---|
| S1 | 1.88(0.20) | 2.84(0.52) | 1.82(0.47) | 2.00(0.24) | 2.04(0.42) | 2.40(0.32) | 1.01(0.17) | 0.74(0.15) | 0.69(0.06) |
| S2 | 2.56(0.11) | 3.42(0.40) | 2.86(0.36) | 2.54(0.20) | 2.50(0.34) | 3.12(0.76) | 0.99(0.33) | 0.95(0.04) | 0.78(0.11) |
| S3 | 3.06(0.06) | 2.84(0.48) | 3.18(0.36) | 6.05(0.22) | 3.60(0.70) | 3.34(1.14) | 0.91(0.28) | 0.93(0.36) | 0.84(0.23) |
| S4 | 2.90(0.28) | 2.64(0.38) | 1.79(0.65) | 2.66(0.14) | 4.82(0.62) | 2.06(0.12) | 2.03(0.50) | 1.84(0.74) | 1.76(0.16) |

S1, lightly polluted soil; S4, highly polluted soil; S2 and S3 intermediate polluted soils; data shown as mean (SD) ($n$=5 for first six crops, 3 for last three crops)

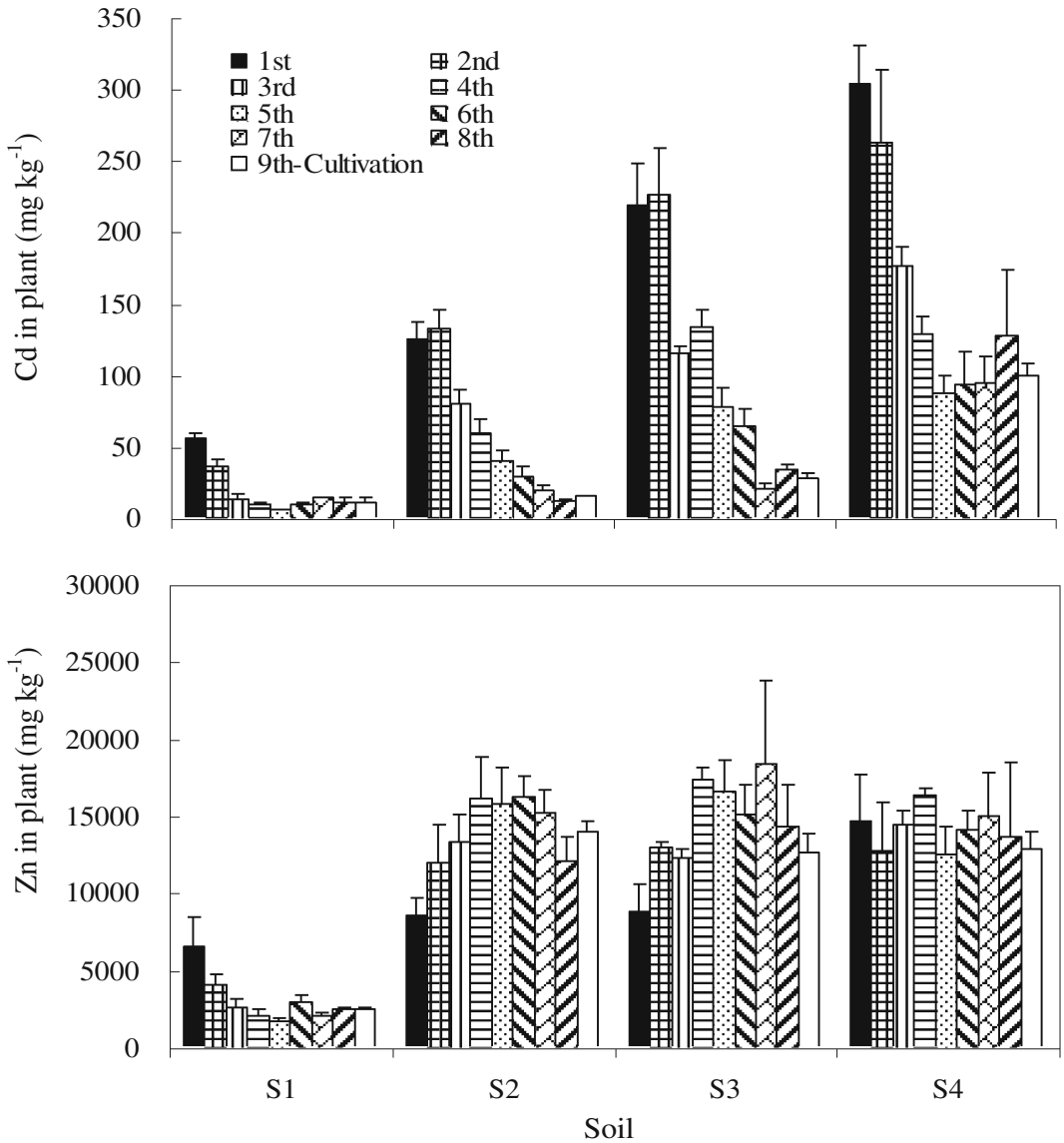


**Fig. 1** Concentrations of Cd and Zn in shoots of plants of each crop grown in four contaminated soils. S1, lightly polluted soil; S2 and S3 intermediate polluted soils; S4, highly polluted soil. Error bars denote standard deviation ($n$=5 for first six crops, 3 for last three crops)

## Changes in soil *aqua regia*-extractable cadmium and zinc

The concentrations of *aqua regia*-extractable Cd and Zn in the four contaminated soils with and without phytoextraction, and changes in the concentrations after phytoextraction (metal concentrations in soils without phytoextraction minus metal removal by plants) are presented in Fig. 2. Compared with the unplanted controls, after phytoextraction both *aqua regia*-extractable Cd and Zn had decreased greatly and the extents of decline in S1, S2, S3 and S4 were 89.1, 96.8, 90.1, and 80.1 % for Cd, and 64.2, 70.1, 32.0, and 24.8 % for Zn, respectively. Comparing the determined soil metal and the calculated metal removed by the plants, the decrease in metal concentration in soil after phytoextraction can be explained by plant uptake (Fig. 2).

*Aqua regia*-extractable Cd and Zn in the different soil particle size fractions were determined with three replicates for the first three size fractions, no replicates for colloidal and nanocolloidal fractions and the analytical error of the replicates did not exceed 5 %. The data are shown in Table 4. Cd and Zn in contaminated soil increased with decreasing soil particle size, except for Cd and Zn in the nanocolloid particles. Briefly, Cd and Zn concentrations in whole soil were higher than in the fine sand and silt fractions and lower than in the other three size fractions (Table 4). Plant uptake resulted in a clear decrease in Cd and Zn and the decrease in absolute concentration was more pronounced with declining soil particle size (Table 4). The amounts of Cd and Zn in the different soil particle size fractions before and after phytoextraction were calculated by multiplying the metal concentrations by fraction weight (Fig. 3). Because the coarse fractions had much more weight percentage than did the fine fractions (see section 2.2), most of the Cd and Zn occurred in the first three particle fractions and the decreases in Cd and Zn in the coarse particle fractions after phytoextraction were much larger than in the fine fractions, following the sequence: fine sand, silt and fine silt > colloids > nanocolloids.

## Changes in the chemical speciation of cadmium and zinc

Most of the soil Cd and Zn without phytoextraction was partitioned in the acid-soluble and reducible fractions which accounted for over 90 % of the metals, but Zn in the slightly polluted soil (S1) occurred mainly in the acid-soluble fraction, followed by the residual, oxidisable and then reducible fractions (Table 5). After phytoextraction the acid-soluble fraction showed the largest decrease, followed by the reducible fraction and then the oxidisable and residual fractions (Table 5).

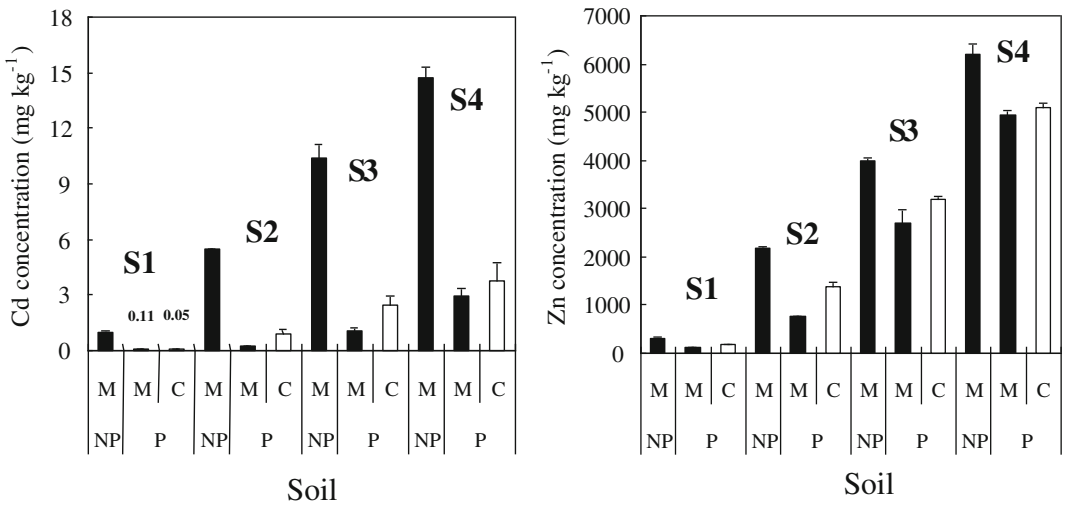


**Fig. 2** Concentrations of total measured (M) Zn and Cd in four contaminated soils without phytoextraction (NP), and after nine (P) crops of phytoextration (filled box) and the calculated (C) concentrations (difference between metal in "unplanted" soil and metal removal by plant) after nine crops of phytoextraction (blank box). S1, lightly polluted soil; S2 and S3 intermediate polluted soils; S4, highly polluted soil. ($n$=5 for first six crops, 3 for last three crops). Error bars denote standard deviation ($n$=5 for first six crops, 3 for last three crops)

**Table 4** Concentrations of *aqua regia*-extractable Cd and Zn in different particle-size fractions after phytoextraction experiment (mg $kg^{-1}$)

| Particle size range (μm) | S1 | | | | S4 | | | |
|---|---|---|---|---|---|---|---|---|
| | Cd | | Zn | | Cd | | Zn | |
| | Planted | Unplanted | Planted | Unplanted | Planted | Unplanted | Planted | Unplanted |
| Whole soil | 0.11 | 1.01 | 111 | 310 | 2.92 | 14.7 | 4,952 | 6,191 |
| Fine sand (50–250) | 0.09 | 0.53 | 87.5 | 187 | 2.11 | 12.4 | 2,999 | 4,451 |
| Silt (5–50) | 0.18 | 0.81 | 110 | 237 | 2.36 | 11.5 | 3,141 | 4,849 |
| Fine silt (1–5) | 0.42 | 1.67 | 269 | 618 | 4.22 | 27.3 | 8,589 | 12,283 |
| Colloids (0.1–1) | 0.90 | 2.70 | 482 | 903 | 6.51 | 33.4 | 12,712 | 17,801 |
| Nanocolloids (< 0.1) | 0.32 | 4.25 | 430 | 887 | 6.88 | 33.2 | 14,557 | 17,938 |

"Unplanted" and "Planted" denote soil without phytoextraction and with nine crops of phytoextraction respectively; S1, lightly polluted soil; S4, highly polluted soil

In common with whole soil, the metals in the particle size fractions were mainly fractionated in the first two extraction steps (except for Zn in S1) (Fig. 4). For the soils without phytoextraction, the percentage of the metals

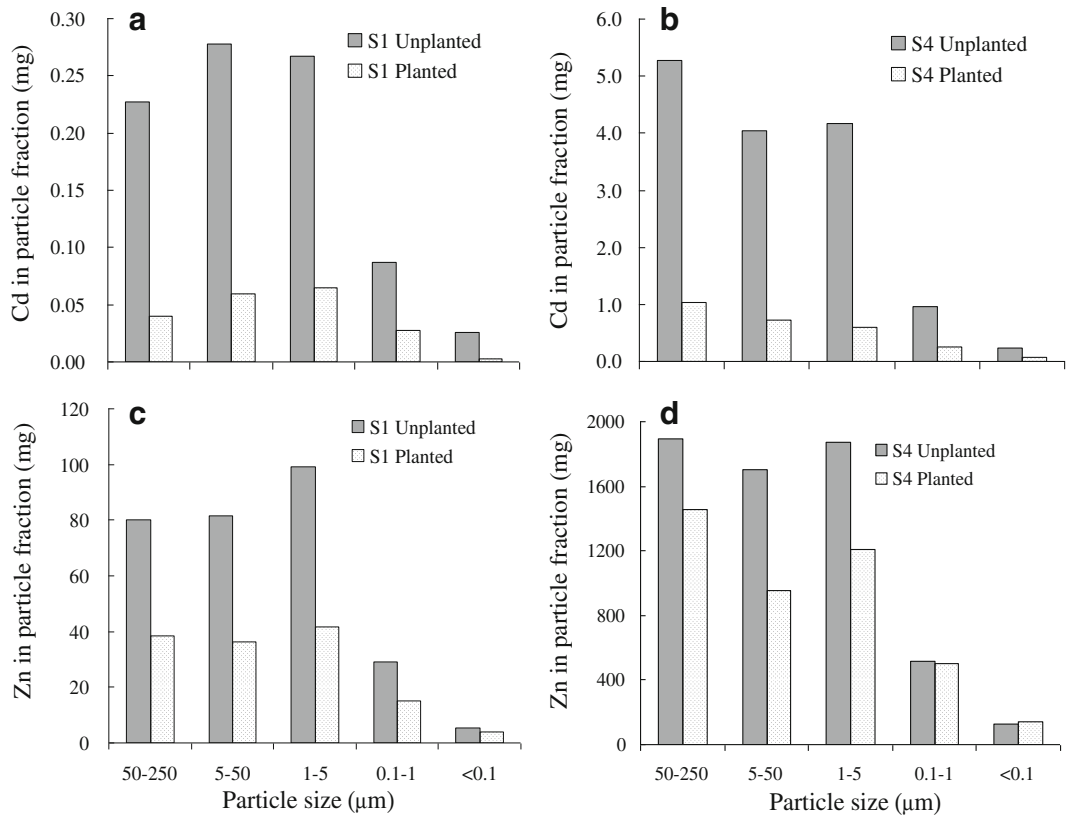


**Fig. 3** Cd (A and B) and Zn (C and D) contents of different particle fractions of the two soils before and after phytoextraction. Data were obtained by multiplying the mass of each fraction in one kilogram of whole soil by the metal concentrations; S1, lightly polluted soil; S4, highly polluted soil; "Planted", soil with nine crops of phytoextraction; "Unplanted", soil without phytoextraction

**Table 5** Chemical speciation of Cd and Zn (mg $kg^{-1}$) in the contaminated soils after nine consecutive crops of *S. plumbizincicola*

| Soil | Plant treatment | Acid soluble | Reducible | Oxidisable | Residual |
|---|---|---|---|---|---|
| Cd | | | | | |
| S1 | Unplanted | 0.49(0.03) | 0.46(0.06) | 0.02(0.00) | 0.02(0.01) |
| | Planted | 0.04(0.00) | 0.06(0.01) | 0.01(0.00) | 0.02(0.01) |
| | Decrease, % | 91.8* | 87.0* | 50.0* | 0.0 |
| S2 | Unplanted | 3.29(0.10) | 1.76(0.13) | 0.10(0.01) | 0.05(0.00) |
| | Planted | 0.07(0.01) | 0.10(0.01) | 0.02(0.00) | 0.05(0.00) |
| | Decrease, % | 97.9* | 94.3* | 80.0* | 0.0 |
| S3 | Unplanted | 7.07(0.05) | 2.98(0.11) | 0.27(0.01) | 0.08(0.01) |
| | Planted | 0.48(0.13) | 0.38(0.02) | 0.16(0.07) | 0.06(0.00) |
| | Decrease, % | 93.2* | 87.2* | 40.7* | 25.0 |
| S4 | Unplanted | 10.5(0.4) | 3.00(0.07) | 0.27(0.13) | 0.11(0.01) |
| | Planted | 1.65(0.07) | 0.96(0.06) | 0.19(0.02) | 0.18(0.12) |
| | Decrease, % | 84.3* | 68.0* | 29.6 | −63.6 |
| Zn | | | | | |
| S1 | Unplanted | 90.7(4.7) | 63.9(1.2) | 47.8(4.3) | 79.8(7.0) |
| | Planted | 6.48(1.46) | 6.34(0.90) | 44.5(11.4) | 68.7(3.2) |
| | Decrease, % | 92.9* | 90.1* | 6.9 | 13.9 |
| S2 | Unplanted | 1,135(125) | 696(38) | 198(23) | 103(1) |
| | Planted | 255(9) | 212(13) | 159(75) | 104(1) |
| | Decrease, % | 77.5* | 69.5* | 19.7 | −1.0 |
| S3 | Unplanted | 2,719(69) | 1,257(29) | 236(16) | 136(12) |
| | Planted | 1,258(134) | 869(103) | 224(12) | 137(7) |
| | Decrease, % | 53.7* | 30.9* | 5.1 | −0.7 |
| S4 | Unplanted | 4,139(170) | 1,734(7) | 346(37) | 145(18) |
| | Planted | 2,660(95) | 1,461(262) | 335(27) | 142(4) |
| | Decrease, % | 35.7* | 15.7* | 3.2 | 2.1 |

"Unplanted" and "Planted" denote soil without phytoextraction and with nine crops of phytoextraction respectively; S1, lightly polluted soil; S2 and S3 intermediate polluted soils; S4, highly polluted soil. Metal concentration shown as mean(SD) ($n$=3); the relative decrease in metals calculated by (Unpanted-Planted)/Uplanted×100; "*" in the "Decrease, %" denotes a significant difference between Unplanted and Planted

in the acid soluble fraction decreased and in the reducible fraction increased with decreasing particle size. However, the percentage of the sum of acid soluble and reducible fractions changed little with particle size variation in both soils (Fig. 4). Moreover, in S1 the percentage of Zn in the residual fraction increased with decreasing particle size and became dominant in the finer particles (Fig. 4c). After phytoextraction, the percentage of acid soluble Cd in each size fraction decreased except for the colloidal fraction of S1; the reducible fraction showed much more complicated changes, and other two fractions shown slight increases (Fig. 4a and b). For Zn, after repeated phytoextraction the percentage of Zn in the acid soluble and reducible fraction of S1 decreased and the other two fractions increased; in soil S4 a decrease in Zn percentage in acid soluble and an increase in the other three fractions were observed (Fig. 4c and d).

## Discussion

### Plant metal uptake during repeated phytoextraction

Metal uptake from the soil by plants depends largely on both plant physiological properties (e.g. metal absorption by the roots, translocation and accumulation in the shoots) and the soil metal availability. Hyperacumulators have a very strong ability to take up and accumulate metals in their shoots (Rascio and Navari-Izzo 2011). Thus, during phytoextraction using hyperaccumulators, soil metal availability could be the main factor limiting metal uptake. Soil properties such as soil metal concentration, pH and soil components will control soil metal bioavailability (Kirkham 2006). Plants can decrease metal availability because of plant removal of metals from the soil and can also enhance metal availability by the production of root exudates (Degryse et al. 2008). In the present study, metal

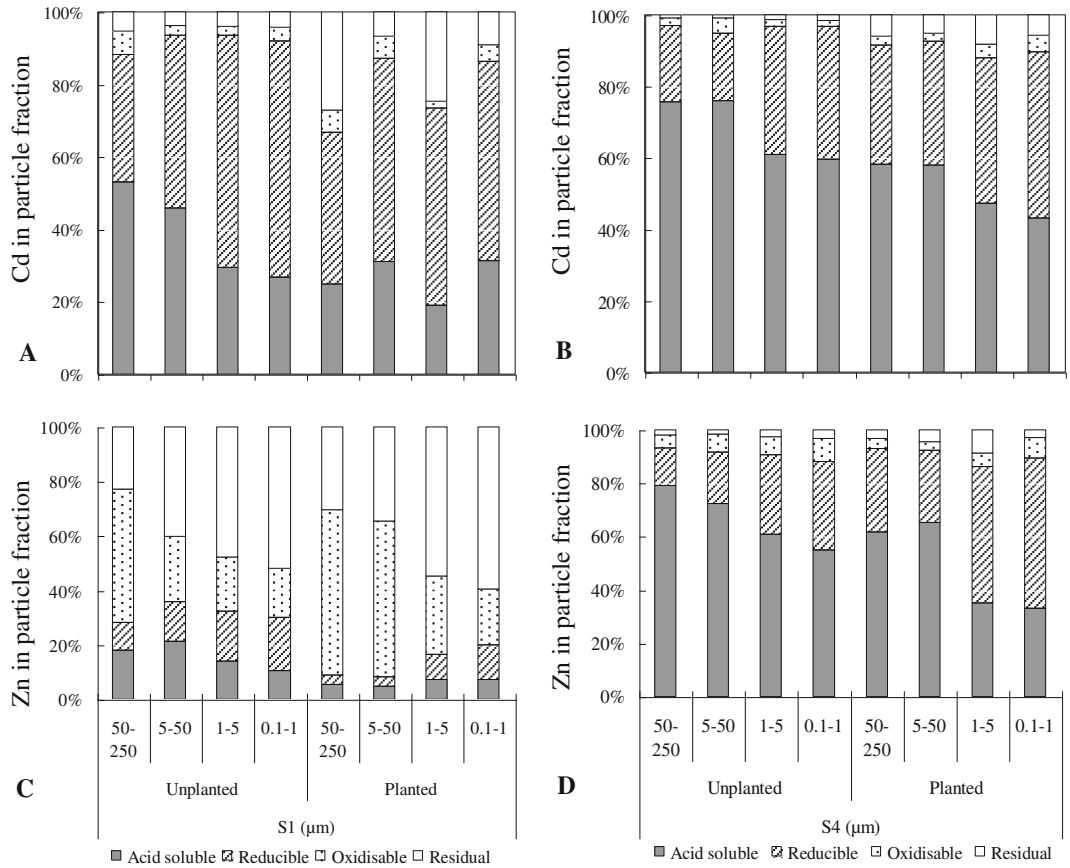


**Fig. 4** Percentage distribution of Cd (A and B) and Zn (C and D) in different size soil fractions before and after repeated phytoextraction. S1, lightly polluted soil; S4, highly polluted soil; "Planted", soil with nine crops of phytoextraction; "Unplanted", soil without phytoextraction

uptake by the Cd/Zn hyperaccumulator depended mainly on the soil properties and interactions between plants and soils. During the repeated phytoextraction with sequential harvest times, a decrease in shoot Cd was found in all four soils and for Zn in slightly polluted soil S1 (Fig. 1). This may be related to a decline in soil metal availability during repeated phytoextraction as indicated by the reduction in acid-soluble and reducible soil metal fractions after phytoextraction (Table 5).

Unexpectedly, plant Zn in S2 and S3 increased slightly in the early crops and showed similar plant Zn in S4 during repeated phytoextraction (Fig. 1). This is inconsistent with a decrease in soil metal during phytoextraction. In addition, similar shoot Zn concentrations were found in S2, S3 and S4 and this is also inconsistent with the different Zn levels in these three soils (Figs. 1 and 2). Furthermore, similar shoot Zn in S1 and similar shoot Cd especially in S1 and S4 were also found in the later few crops of the phytoextraction sequence (Fig. 1). These results indicate that in the above cases the plants maintained a steady level of metal uptake during repeated phytoextraction. However, this type of plant control cannot result from limitation of plant metal uptake ability because a previous study showed that *S. plumbizincicola* can accumulate up to 30,000–40,000 mg $kg^{-1}$ Zn in the shoots when the plants grow in nutrient solution with 600 μM Zn concentration for 56 days (Li et al. 2010). In another study we also found similar plant Cd (about 100 mg $kg^{-1}$) and Zn (4,000 mg $kg^{-1}$) concentrations during repeated phytoextraction when this plant species grew in an alkaline soil (soil pH 8.00, soil Cd 16.9 and Zn 1,308 mg $kg^{-1}$), although the availability of Cd and Zn decreased by over 88 % (evaluated by the technique of diffusive gradients in thin films, DGT) after phytoextraction (in preparation). There are two possible explanations. Firstly, the rhizosphere activity of the hyperaccumulator may increase metal availability by root exudates (Degryse et al. 2008; Li et al. 2011). In the case of low metal concentrations in the soil solution and relative large metal potential pool size

(e.g. a soil with high pH and high metal concentrations), metal released from the potential pool will be much higher with the root exudates than the metal released without these materials (Degryse et al. 2008). In other words, the rhizosphere activity may make a major contribution to metal uptake. In addition, root foraging for metal has been demonstrated for some hyperaccumulators (Liu et al. 2010) and this may also play a role in maintaining plant metal concentrations. Secondly, the plants themselves may control root uptake and/or root-to-shoot translocation of metals. The processes by which metals enter root cells and are translocated from root to shoot in hyperaccumulators mostly involve the metal trans-membrane transporter proteins (Rascio and Navari-Izzo 2011). Assuncao et al (2001) reported that the expression of metal transporter in *Thlaspi caerulescens* is related to Zn and a calamine soil induced much higher transporter (ZTP1) expression in the plant than did serpentine or other soils. This may indicate that a range of metal concentrations in the plant growth environment can induce certain amounts of transporter proteins in plants and thus regulate shoot metal concentrations. Although the final explanation still needs to be validated, plant Cd especially in S1 and S4 and plant Zn in S1 at the later stages of repeated phytoextraction and plant Zn in S2, S3, and S4 did not change with changes in soil metal showing marked differences in metal availability and with decreasing metal availability during phytoextraction. This indicates that the hyperaccumulator itself has a role in maintaining or controlling soil plant-available metals.

### *Aqua regia*-extractable metals in whole soil and in different particle size fractions

After phytoextraction by nine consecutive crops, *aqua regia*-extractable Cd and Zn had decreased greatly at all soil pollution levels (Fig. 2). The removal efficiencies of Cd were high with a minimum of 80.1 (S4) and a maximum of 96.8 % (S2) (Fig. 2). The removal efficiencies of Zn were also high at relatively low levels of soil pollution (S1 and S2), but in the more highly polluted soils (S3 and S4) the removal efficiencies were low. This demonstrates the successful phytoremediation of the metal-contaminated soil by the hyperaccumulator, but for successful phytoextraction of soils highly polluted with Zn it may be necessary to apply other remediation methods together with phytoextraction.

Because of their much higher soil cation exchange capacities (CEC) and organic matter contents and the presence of Fe-Mn-Al oxides (Table 2), the finer soil particles adsorbed more Cd and Zn than did the coarse particles (Table 4) and this is consistent with previous studies (Lair et al. 2007; Tang et al. 2009). Different metal contents and affinities for metals in soil particle fractions would result in different metal availability to the plants (Madrid et al. 2008). The smaller particles can enhance plant uptake of Cd and Zn due to their high specific area by providing greater root-particle contact (Chen et al. 2008). In our study plant metal uptake resulted in large decreases in Cd and Zn concentrations in all soil particle size fractions, and the decrease in absolute concentration was more pronounced with decreasing particle size, an effect which may be associated with high bioavailability of metals in the fine particles (Madrid et al. 2008). In the lightly polluted soil (S1) the relative decrease in metals was greater in the nanocolloids than in the colloids (especially in the case of Cd) but this was not found in the highly polluted soil (S4) (Table 4). These different results for the two soils may be explained by the solubility of nanocolloids in the soil solution and their strong adsorption capacity for metals. In S1 when metals in solution were taken up by the plants and the supply from the solid phase was inadequate, the metals in the nanocolloids were more readily utilized by the plants than the metals in the other fractions because they were more accessible to the roots (readily mobile in the soil solution). In S4 the decrease in metals in solution taken up by the plants was supplemented by the labile fraction of the coarse particles, and less change in the metals in the nanocolloids was induced by the plants.

Although the decreases in Cd and Zn concentrations after phytoextraction were more pronounced in the fine particles than in the coarse fraction, when the mass of each particle size fraction was considered the fine sand, silt and fine silt made a much greater contribution to the decrease in Cd and Zn in whole soil during plant uptake than did the colloidal and nanocolloidal fractions (Fig. 3). This suggests that the coarse particle fractions may make a much greater contribution to metal decrease induced by plant uptake than the fine particles.

### Speciation of heavy metals in soil and in particle fractions

The metal fractions extracted by the BCR (or modified BCR) method at different steps represented different degrees of bioavailability for the different forms of

association and different mechanisms of availability (Anju and Banerjee 2010). The metal extracted at the first step is regarded as exchangeable, water and acid-soluble, at the second step as Mn/Fe oxides-bound, and at the third step as oxidisable organic matter- and sulphides-bound, and the residual fraction is regarded as metal in the crystal lattice of minerals (Hass and Fine 2010).

The Cd in the different BCR fractions was distributed mainly in the first two extracts, and the sum of the acid-soluble and reducible fractions comprised over 90 % of the total Cd concentration in non-remediated soil (Table 5). This is consistent with other studies (Anju and Banerjee 2010; Li and Thornton, 2001). This distribution indicates that Cd tends to be fixed by electrostatic attraction, by carbonates, and by Fe/Mn oxides rather than by organic matter and sulphides. Similarly, Zn in S4 also occurred mainly in the first two fractions extracted but in S1 a large proportion of Zn was present in the residual fraction. This may be due to the small amount of exogenous Zn in S1, with the residual fraction consisting mainly of background Zn in soil crystal lattices. After repeated phytoextraction the Cd and Zn concentrations had decreased greatly in the acid soluble fraction and then the reducible fraction, but the oxidisable and residual fractions changed little (Table 5). This indicates that acid-soluble Cd and Zn was the most plant-available followed by the forms bound to Fe/Mn oxides (reducible fraction), and these two metal fractions can be removed by phytoextraction, but the oxidisable and residual fractions were the least available to the plants. Introducing hydrogen ions into the soil to release the acid soluble metal fraction or changing the soil redox status intermittently and thus re-balancing the reducible metal fractions might be effective methods to enhance phytoextraction efficiency, especially of highly polluted soils.

BCR extraction procedures were also used to evaluate Cd and Zn availability in the different particle size fractions. As particle size declined the percentage of acid soluble metal fraction decreased and the reducible fraction increased (Fig. 4), possibly indicating that Fe/Mn oxides in the fine size fractions play important roles in adsorbing metals, a conclusion which is supported by the presence of more Fe/Mn oxides in the finer size fractions than in the coarse fractions (Table 2). After phytoextraction the percentage of acid soluble and reducible metal fractions in all size fractions of S1 decreased, but in S4 only the percentage of acid soluble metal decreased and the reducible fraction increased (Fig. 4). This further indicates that the acid-soluble metal fractions were much more available to the plants than the reducible fractions, and that re-balancing the reducible metal fraction might enhance phytoextraction efficiency.

## Conclusions

During the repeated phytoextraction plant Cd uptake decreased with each harvest in turn in all soils and Zn uptake also declined in S1. However, similar shoot Cd in S1 and S4, and similar shoot Zn in S1 in the later stages of phytoextraction, and similar shoot Zn in S2, S3 and S4 were found although metal availability differed in soils and decreased during phytoextraction. This indicates that the plant has some ability to control metal uptake. After repeated phytoextraction both soil Cd and Zn had decreased greatly, and the sequential extraction (BCR) indicates that acid soluble Cd and Zn is the fraction most available to the hyperaccumulator followed by reducible, and then oxidizable and residual fractions.

Although after phytoextraction the decreases in Cd and Zn concentrations were enhanced with decreasing the particle size, when the mass of each particle fraction was taken into consideration the coarse fractions made a much greater contribution to the decrease in Cd and Zn in whole soil than did the colloidal and nano-colloidal fractions during plant uptake.

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